

## WHAT IS CLAIMED IS:

1. A method of identifying, analyzing or typing a polymorphic DNA fragment in a sample of DNA, said method comprising contacting said sample of DNA with one or more DNA polymerases substantially reduced in the ability to add one or more non-templated nucleotides to the 3' terminus of a DNA molecule, amplifying said polymorphic DNA fragment within said sample and analyzing said amplified polymorphic DNA fragment.

2. A method of producing amplified copies of a polymorphic DNA fragment which comprise substantially no non-templated 3' terminal nucleotides, said method comprising contacting a DNA sample with one or more DNA polymerases substantially reduced in the ability to add one or more non-templated nucleotides to the 3' terminus of a DNA molecule and amplifying said polymorphic DNA fragment within said DNA sample.

3. A method of cloning a DNA molecule comprising contacting said DNA molecule with one or more DNA polymerases substantially reduced in the ability to add one or more non-templated nucleotides to the 3' terminus of a DNA molecule, amplifying said DNA molecule and inserting said DNA molecule into a vector.

4. The method of claim 3, wherein said vector is blunt-ended.

5. The method of claim 1, wherein said polymorphic DNA fragment is selected from the group of polymorphic DNA fragments comprising a minisatellite DNA fragment, a microsatellite DNA fragment and a STR DNA fragment.

6. The method of claim 1, wherein said polymerases are thermostable DNA polymerases.

7. The method of claim 6, wherein said thermostable DNA polymerases are *Thermotoga* DNA polymerases and mutants or derivatives thereof.

8. The method of claim 7, wherein said DNA polymerase is a *Tne* or *Tma* DNA polymerase.

9. The method of claim 1, wherein said DNA polymerases are substantially reduced in 3'-5' exonuclease activity.

10. The method of claim 1, wherein said DNA polymerases are substantially reduced in 5'-3' exonuclease activity.

11. The method of claim 9, wherein said DNA polymerases are substantially reduced in 5'-3' exonuclease activity.

12. The method of claim 1, wherein said DNA polymerases contain one or more modifications or mutations which reduce the ability of the polymerase to add one or more non-templated 3' nucleotides to a synthesized nucleic acid molecule.

13. The method of claim 12, wherein said DNA polymerases are substantially reduced in at least one activity selected from the group consisting of:

- (a) 3'-5' exonuclease activity; and
- (b) 5'-3' exonuclease activity.

14. The method of claim 13, wherein said polymerases have substantially reduced 3'-5' exonuclease and 5'-3' exonuclease activity.

15. The method of claim 13, wherein said polymerase is substantially

reduced in 3'-5' exonuclease activity.

16. The method of claim 12, wherein said polymerases comprise one or more mutations or modifications in the O-helix of said polymerase.

17. The method of claim 16, wherein said O-helix is defined as RXXXXXXXFXXXYX (SEQ ID NO:11), wherein X is any amino acid.

18. The method of claim 17, wherein said mutation or modification is at position R (Arg) and/or F (Phe) and/or K (Lys) of said O-helix or combinations thereof.

20. The method of claim 16, wherein said mutation or modification is an amino acid substitution at position R and/or F and/or K of said O-helix or combinations thereof.

19. The method of claim 1, wherein said polymerase is selected from the group consisting of:

*Tne* N'Δ219, D323A;

*Tne* N'Δ283, D323A;

*Tne* N'Δ284, D323A;

*Tne* N'Δ193, D323A;

*Tne* D137A, D323A;

*Tne* D8A, D323A;

*Tne* G195D, D323A;

*Tne* G37D, D323A;

*Tne* N'Δ283;

*Tne* D137A, D323A, R722K;

*Tne* D137A, D323A, R722Y;

*Tne* D137A, D323A, R722L;

*Tne* D137A, D323A, R722H;

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*The* D137A, D323A, R722H, F730Y;

*Tne* D137A, D323A, R722H, K726R;

*The* D137A, D323A, R722H, K726H;

*The* D137A, D323A, R722Q, F730Y;

*The* D137A, D323A, R722Q, K726R;

*The* D137A, D323A, R722Q, K726H;

*The* D137A, D323A, R722N, F730Y;

*The* D137A, D323A, R722N, K726R;

*The* D137A, D323A, R722N, K726H;

*The* D137A, D323A, F730S;

*The N'Δ283, D323A, R722K/H/Q/N/Y/L;*

*The* N'Δ219, D323A, R722K;

*The* N'Δ219, D323A, F730Y;

*The* N $\Delta$ 219, D323A, K726R;

*The N'Δ219, D323A, K726H;*

*The* D137A, D323A, F730S, R722K/Y/Q/N/H/L, K726R/H;

*The* D137A, D323A, F730T, R722K/Y/Q/N/H/L, K726R/H;

*The* D137A, D323A, F730T;

*The F730S;*

*The F730A;*

*The K726R;*

The K726H; and

*The* D137A, D323A, R722N.

21. A method of determining the relationship between a first individual and a second individual, said method comprising comparing a population of amplified DNA molecules in a sample of DNA from said first individual to that of said second individual prepared according to the method of claim 1.

5 22. The method of claim 21, wherein said sample of DNA from said first individual is a known sample and said sample of DNA from said second individual is an unknown sample.

10 23. A kit for the identification, analysis or typing of a polymorphic DNA fragment, said kit comprising one or more DNA polymerases substantially reduced in the ability to add one or more non-templated nucleotides to the 3' terminus of a DNA molecule.

15 24. The kit of claim 23, said kit further comprising one or more components selected from the group consisting of one or more DNA primers, one or more deoxynucleoside triphosphates, and a buffer suitable for use in the identification, analysis or typing of a polymorphic DNA fragment.

25. The kit of claim 23, wherein said polymerases are thermostable DNA polymerases.

26. The kit of claim 25, wherein thermostable DNA polymerases are *Thermotoga* DNA polymerases.

20 27. The kit of claim 23, wherein said DNA polymerase is substantially reduced in 3'-5' exonuclease activity.

28. The kit of claim 23, wherein said DNA polymerase is substantially reduced in 5'-3' exonuclease activity.

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29. The kit of claim 23, wherein said DNA polymerases comprise one or more modifications or mutations which reduce the ability of the polymerase to add one or more non-templated 3' nucleotides to a synthesized nucleic acid molecule.

5 30. The kit of claim 29, wherein said polymerases comprise one or more mutations in the O-helix of said polymerase.

31. The kit of claim 30, wherein said O-helix is defined as RXXXXXXXFXXXYYX (SEQ ID NO:11), wherein X is any amino acid.

10 32. The kit of claim 31, wherein said mutation or modification is at position R (Arg) and/or F (Phe) and/or K (Lys) of said O-helix or combinations thereof.

33. The method of claim 31, wherein said mutation or modification is an amino acid substitution at position R and/or F and/or K of said O-helix or combinations thereof.

15 34. A polymerase which has been modified or mutated to reduce, substantially reduce or eliminate the ability of the polymerase to add non-templated 3' nucleotides to a synthesized nucleic acid molecule.

35. The polymerase of claim 34, wherein said polymerase is a DNA or RNA polymerase.

20 36. The polymerase of claim 34, wherein said polymerase is substantially pure.

37. The polymerase of claim 34, wherein said polymerase is mesophilic or thermostable.

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38. The polymerase of claim 34, wherein said polymerase is selected from the group consisting of *Tne* DNA polymerase, *Taq* DNA polymerase, *Tma* DNA polymerase, *Tth* DNA polymerase, *Tli* DNA polymerase, VENT™ DNA polymerase, *Pfu* DNA polymerase, DEEPVENT™ DNA polymerase, *Pwo* DNA polymerase, *Bst* DNA polymerase, *Bca* DNA polymerase, *Tfi* DNA polymerase, and mutants, variants and derivatives thereof.

39. The polymerase of claim 34, wherein said polymerase is substantially reduced in at least one activity selected from the group consisting of:

- (a) 3'→5' exonuclease activity; and
- (b) 5'→3' exonuclease activity.

40. The polymerase of claim 39, wherein said polymerase is substantially reduced in 3'-5' exonuclease activity.

41. The polymerase of claim 39, wherein said polymerase is substantially reduced in 5'-3' exonuclease activity.

42. The polymerase of claim 41, which is modified or mutated to reduce or eliminate 3'-5' exonuclease activity.

43. The polymerase of claim 34, which comprises one or more modifications or mutations in the O-helix of said polymerase.

44. The polymerase of claim 43, wherein said O-helix is defined as RXXXXXXXFXXXYY (SEQ ID NO:11), wherein X is any amino acid.

45. The polymerase of claim 44, wherein said mutation or modification is at position R (Arg) and/or F (Phe) and/or K (Lys) of said O-helix or combinations thereof.

46. The polymerase of claim 44, wherein said mutation or modification is an amino acid substitution at position R and/or F and/or K of said O-helix or combinations thereof.

47. The polymerase of claim 46, wherein R (Arg) is substituted with an amino acid selected from the group consisting of Ala, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Phe, Pro, Ser, Thr, Trp, Try and Val.

49. The polymerase of claim 46, wherein F (Phe) is substituted with an amino acid selected from the group consisting of Ala, Asn, Arg, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Lys, Met, Pro, Ser, Thr, Trp, Try and Val.

50. The polymerase of claim 46, wherein K (Lys) is substituted with an amino acid selected from the group consisting of Ala, Arg, Asn, Asp, Cys, Gln, Glu, Gly, His, Ile, Leu, Met, Phe, Pro, Ser, Thr, Trp, Try and Val.

51. The polymerase of claim 46, wherein K (Lys) is substituted with Arg or His.

52. A mutant *The* DNA polymerase protein selected from the group consisting of:

*Tne* N'Δ219, D323A;

*Tne* N'Δ283, D323A;

*Tne* N'Δ284, D323A;

*Tne* N'Δ193, D323A;

*Tne* D137A, D323A;

*Tne* D8A, D323A;

*Tne* G195D, D323A;

*Tne* G37D, D323A;

*Tne* N'Δ283;

*Tne* D137A, D323A, R722K;



*The* D137A, D323A, R722Y;  
*The* D137A, D323A, R722L;  
*The* D137A, D323A, R722H;  
*The* D137A, D323A, R722Q;  
5 *The* D137A, D323A, F730Y;  
*The* D137A, D323A, K726R;  
*The* D137A, D323A, K726H;  
*The* D137A, D323A, R722K, F730Y;  
*The* D137A, D323A, R722K, K726R;  
10 *The* D137A, D323A, R722K, K726H;  
*The* D137A, D323A, R722H, F730Y;  
*The* D137A, D323A, R722H, K726R;  
*The* D137A, D323A, R722H, K726H;  
*The* D137A, D323A, R722Q, F730Y;  
15 *The* D137A, D323A, R722Q, K726R;  
*The* D137A, D323A, R722Q, K726H;  
*The* D137A, D323A, R722N, F730Y;  
*The* D137A, D323A, R722N, K726R;  
*The* D137A, D323A, R722N, K726H;  
20 *The* D137A, D323A, F730S;  
*The* N'Δ283, D323A, R722K/H/Q/N/Y/L;  
*The* N'Δ219, D323A, R722K;  
*The* N'Δ219, D323A, F730Y;  
*The* N'Δ219, D323A, K726R;  
25 *The* N'Δ219, D323A, K726H;  
*The* D137A, D323A, F730S, R722K/Y/Q/N/H/L, K726R/H;  
*The* D137A, D323A, F730T, R722K/Y/Q/N/H/L, K726R/H;  
*The* D137A, D323A, F730T;  
*The* F730S;  
30 *The* F730A;  
*The* K726R;

*Tne* K726H; and  
*Tne* D137A, D323A, R722N.

53. A vector comprising a gene encoding the polymerase of claim 34.

5 54. The vector of claim 53, wherein said gene is operably linked to a promoter.

55. The vector of claim 54, wherein said promoter is selected from the group consisting of a  $\lambda$ -P<sub>L</sub> promoter, a *tac* promoter, a *trp* promoter, and a *trc* promoter.

56. A host cell comprising the vector of claim 53.

10 57. A method of producing a polymerase, said method comprising:

- (a) culturing the host cell of claim 56;
- (b) expressing said gene; and
- (c) isolating said polymerase from said host cell.

58. A method of synthesizing a nucleic acid molecule comprising:

- 15 (a) mixing a nucleic acid template with one or more polymerases of claim 34; and
- (b) incubating said mixture under conditions sufficient to make a nucleic acid molecule complementary to all or a portion of said template.

20 59. The method of claim 58, wherein said mixture further comprises one or more nucleotides selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, ddATP, ddCTP, ddGTP, ddITP, ddTTP, [ $\alpha$ -S]dATP, [ $\alpha$ -S]dTTP, [ $\alpha$ -S]dGTP, and [ $\alpha$ -S]dCTP.

60. The method of claim 59, wherein one or more of said nucleotides

are detectably labeled.

61. A method of sequencing a DNA molecule, comprising:

(a) hybridizing a primer to a first DNA molecule;

(b) contacting said DNA molecule of step (a) with deoxyribonucleoside triphosphates, one or more DNA polymerases of claim 34, and a terminator nucleotide;

(c) incubating the mixture of step (b) under conditions sufficient to synthesize a random population of DNA molecules complementary to said first DNA molecule, wherein said synthesized DNA molecules are shorter in length than said first DNA molecule and wherein said synthesized DNA molecules comprise a terminator nucleotide at their 3' termini; and

(d) separating said synthesized DNA molecules by size so that at least a part of the nucleotide sequence of said first DNA molecule can be determined.

62. The method of claim 61, wherein said deoxyribonucleoside triphosphates are selected from the group consisting of dATP, dCTP, dGTP, dTTP, dITP, 7-deaza-dGTP, dUTP, [ $\alpha$ -S]dATP, [ $\alpha$ -S]dTTP, [ $\alpha$ -S]dGTP, and [ $\alpha$ -S]dCTP.

63. The method of claim 61, wherein said terminator nucleotide is ddTTP, ddATP, ddGTP, ddITP or ddCTP.

64. The method of claim 61, wherein one or more of said deoxyribonucleoside triphosphates is detectably labeled.

65. The method of claim 61, wherein one or more of said terminator nucleotides is detectably labeled.

66. A method for amplifying a double stranded DNA molecule, comprising:

5 (a) providing a first and second primer, wherein said first primer is complementary to a sequence at or near the 3'-termini of the first strand of said DNA molecule and said second primer is complementary to a sequence at or near the 3'-termini of the second strand of said DNA molecule;

10 (b) hybridizing said first primer to said first strand and said second primer to said second strand in the presence of the one or more DNA polymerases of claims 34, under conditions such that a third DNA molecule complementary to said first strand and a fourth DNA molecule complementary to said second strand are synthesized;

(c) denaturing said first and third strand, and said second and fourth strands; and

(d) repeating steps (a) to (c) one or more times.

15 67. A kit for sequencing, amplifying or sequencing a DNA molecule comprising one or more polymerases of claim 34.

68. The kit of claim 67, further comprising one or more dideoxyribonucleoside triphosphates and/or one or more deoxyribonucleoside triphosphates.